MAIZE GENETICS

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THE BEHAVIOR OF "UNSATURATED" BROKEN ENDS OF CHROMOSOMES

In all cases involving rearrangements of segments of chromosomes which give rise to translocations, inversions, deficiencies, etc., it has been necessary to postulate some force that breaks a chromosome and some force that results in the permanent 2-by-2 fusion of the broken ends. Previous investigations in maize on the mitotic behavior of ring-shaped chromosomes had suggested that fusions may occur between two recently broken ends of chromosomes which enter the same nucleus. Such broken ends may be considered "unsaturated," i.e., capable of fusion with similar "unsaturated" broken ends, until fusion with another broken end occurs or until the end loses its capacity for fusion. To determine whether such an "unsaturated" state exists, male gametes containing a chromosome 9 whose short arm had been broken by mechanical pull at the previous anaphase were united with female gametes containing a similar recently broken chromosome 9. The zygote formed received from each gamete nucleus a single chromosome with a single recently broken end. On the basis of published data, these two recently broken ends, derived from separate nuclei, are believed to be in the "unsaturated" state and therefore capable of fusion with each other. If some force exists that brings these unsaturated ends together and results in fusion, a dicentric chromosome should be produced composed of the chromosome 9 contributed by the female gamete and the chromosome 9 contributed by the male gamete, fused at the ends of their short arms. Through the use of the endosperm markers *I* and *C* and through the aberrant mitotic behavior that reflects the presence of such broken chromosomes in the endosperm, it was possible to select the kernels from an ear whose zygote nucleus had received a chromosome with an unsaturated broken end from the male and female gamete nuclei, respectively.

Out of a total of 18,243 kernels examined, 20 non-germless kernels were obviously of the type desired. These kernels were germinated. If fusion had occurred between the broken ends of the chromosomes 9 contributed by the two gametes, following chromosome reduplication, the dicentric chromosome should produce a double anaphase bridge configuration when the two centromeres of each chromatid passed to opposite poles. Breakage of the two bridges would result in the entrance into each nucleus of two newly derived, unsaturated broken ends. Fusion of unsaturated broken ends could occur in each sister telophase nucleus. Again, the two chromosomes 9 would be joined to form one chromosome with two centromeres. Repeated anaphase bridge configurations should be expected to follow from such a chromosomal type of breakage-fusion-bridge cycle. Plants having such a dicentric chromosome and undergoing this cycle should have cells with various types of heterozygous and homozygous duplications and deficiencies of the short arm of chromosome 9 following nonmedian breakages of the anaphase bridges. Because of this process, the plants should be conspicuously modified in appearance. The plants arising from 10 of the 20 kernels were obviously of the type expected if a dicentric chromosome 9 were present. Examination of the early roots confirmed the presence of a dicentric chromosome. Some-

what less than one-half of the anaphase figures showed contiguous double bridges. Owing to death or defective growth of many cells or sectors of tissue, 5 of these plants died in the seedling stage. Four of the remaining 5 plants continued to grow, because sectors of normal-appearing tissues developed. Gradually these sectors gained the ascendancy in growth, until the plant appeared quite normal. The fifth plant produced 3 normal shoots, which arose from the base of the decidedly aberrant and dying main shoot. Microsporocytes were obtained from the 4 recovered plants and from 2 of the 3 recovered shoots of the fifth plant. In all cases, pachytene analysis showed a bivalent chromosome 9. The two chromosomes were not fused at the ends of their short arms. The two broken ends had healed in the ancestor cell which gave rise to the recovered sector. In most cases, the composition of the short arm of each member of the bivalent was greatly modified, although within a tassel sample all examined sporocytes showed the same composition for the individual member of the bivalent. In several of these plants, it was possible to determine the minimum number of fusions, breakages, and bridges which must have occurred before healing of the two broken ends within a single nucleus had occurred. It is likewise known that the compositions of the short arms were entirely different in the sporocytes of the tassels of the 3 recovered shoots of the one original dicentric plant. The two chromosomes 9, however, had maintained their respective derived compositions within each shoot. This indicates that the microsporocyte tissues of each shoot had originated from one individual cell whose cell ancestors had previously been undergoing the chromosomal type of breakage-fusion-bridge cycle involving the original dicentric chromosome 9. The root system responded similarly. In the older roots of the surviving plants, no dicentric anaphase bridge configurations were observed.

These experiments definitely show the existence of an "unsaturated" state of a recently broken end of a chromosome. Owing to causes as yet undetermined, however, such an end may become saturated (healed) without fusion. Following this, the end no longer takes part in any fusions.

The remaining 10 of the original 20 kernels classified as having received a broken chromosome 9 from each parent gave rise to 9 normal-appearing plants and 1 pale-yellow plant which died in the seedling stage. None of these plants showed dicentric bridge configurations in the young roots. Examination of the sporocytes of the 9 surviving plants showed that 4 had received a broken chromosome 9 from each parent; but the morphology of the short arms gave no indication that fusions had occurred between these broken ends. In 1 plant one parent had contributed a broken chromosome 9, but it could not be determined whether the other parent had likewise contributed a broken chromosome 9. In the remaining 4 plants, each parent had contributed a broken chromosome 9, but one broken end had become saturated by fusion with a broken end other than that of the chromosome 9 contributed by the second gamete and possibly before fusion of the gametes themselves. Consequently, healing of the broken end of the second chromosome 9 had occurred. These results indicate that an unsaturated broken end produced by mechanical breakage of an anaphase bridge is capable of fusing with another unsaturated broken end arising from undetermined causes.

A similar type of fusion has likewise been observed in sporocytes of 5 plants which were known to have been derived

from a gametophyte which had received a chromosome 9 with an unsaturated broken end. It is known that mechanical pull caused by an anaphase bridge will frequently break a chromosome at a knob or at the centromere. In 2 of the 5 cases, the centromere of the broken chromosome 9 was fused with the centromere of another chromosome of the complement. In one case, the fused chromosome was composed of the long arm of chromosome 9 and the short arm of chromosome 2. In the second case, it was composed of the long arm of chromosome 9 and the short arm of chromosome 10. In each case, the complementary arm was missing. In three cases, the fusions had occurred at other positions than centromeres. In one case, a segment from the long arm of chromosome 4 had united with the broken end of the short arm of chromosome 9. Since both chromosomes 4 in this plant were completely normal, it is assumed that chromatid fusion in a gametophytic nucleus had occurred between the unsaturated broken end of chromosome 9 and a naturally arising broken end terminating an acentric distal segment of chromosome 4. In the other two cases, both segments of the second broken chromosome were present. Pachytene analysis has led to the following interpretation: In the last two cases mentioned, a break occurred at one position in chromosomes 1 and 8, respectively. In both cases, this resulted in the presence of three unsaturated broken ends in the same nucleus, one of which was the broken end of the short arm of chromosome 9. Fusion occurred between the unsaturated broken end of chromosome 9 and the unsaturated broken end of the acentric segment of the second broken chromosome. This left the centric segment of the second broken chromosome with a single unsaturated broken end, which thereafter healed. This healing of a single unsaturated broken end, when introduced into sporophytic tissues, is in agreement with the results of similar investigations of this behavior.

Phenotypic Effects of Homozygous Deficiencies of Distal Segments of the Short Arm of Chromosome 9

The phenotypic effects in male gametophytes, and in endosperm and sporophytic tissues, of a series of homozygous deficiencies involving distal segments of the short arm of chromosome 9 are being investigated. These deficiencies were obtained through meiotic breakage of a dicentric chromatid 9 which had been produced following crossing over involving a duplicated segment of the short arm of chromosome 9. This method has been previously described (McClintock, 1941; see bibliography). A number of terminal deficiencies have been isolated, ranging in length from a fraction of the terminal chromomere to deficiencies of approximately one-third of the short arm, including the locus of *C*. At pachytene, the short arm of chromosome 9 has approximately 20 chromomeres. Those in the proximal third of the arm are large, those in the distal two-thirds of the arm are small.

The effect of homozygous deficiencies on the functioning of female gametophytes. Plants heterozygous for these deficiencies produce female gametophytes which are totally deficient for the respective segments of chromosome 9. Complete functioning of such gametophytes occurs in all cases of short deficiencies. Only in the case of longer deficiencies which include 4 or more chromomeres is there a reduction in the functioning of such gametophytes. Environmental factors may be involved in this differential functioning. A preliminary test has indicated that, on a single ear, functioning of deficient female

gametophytes may be complete on one day and they may be totally nonfunctional on the succeeding day. Extensive tests are necessary to associate the effect with a particular environmental condition.

The effect of homozygous deficiencies on the appearance and functioning of male gametophytes. Plants heterozygous for these terminal deficiencies produce pollen grains one-half of which carry the deficient chromosome 9. In all cases, homozygous deficient pollen grains are completely filled with starch. Only in the case of deficiencies that include the distal one-third of the short arm is it possible to distinguish any perceptible differences in the appearance of the normal and the homozygous deficient grains. The latter grains appear to be smaller, but an exact identification of each grain has not been possible. Only in the case of distal deficiencies that are greater than one-third of the short arm is there a classifiable visible effect on pollen development. Some starch develops even in pollen grains that are deficient for nearly all of the short arm of chromosome 9.

Pollen grains that are deficient for small terminal segments are completely functional. Those deficient for more than the terminal chromomere, although completely normal in appearance, are nonfunctional.

The phenotypic effects of small terminal deficiencies on endosperm and sporophytic tissues: the deficiency mutants "pale-yellow" and "white" and their dominance relationships. Plants that are heterozygous for small terminal deficiencies produce viable and functional male and female gametophytes. These plants were selfed to determine whether viable endosperms and embryos that were homozygous for these deficiencies could be obtained. In 5 of the 7 cases studied, the endosperm and embryo of kernels having the homozygous deficiencies were completely normal in appearance. In 2 cases, some but not all of the embryos that were homozygous deficient had died before the maturity of the kernel. The endosperm of these kernels, however, was completely normal. In all 5 cases with normal embryo development, pale-yellow seedlings, completely normal in gross morphology and growth rate, grew from these kernels. Although the coleoptiles were light green, little chlorophyll developed in the leaves, and the seedlings died after exhaustion of the food reserves in the endosperm. The surviving embryos in the 2 cases where the homozygous deficiency resulted in early death of some embryos produced white seedlings completely devoid of plastid pigments. Although the gross morphology of these seedlings was normal, the growth rate was considerably retarded. Proof of the association of the pale-yellow and white seedlings with the homozygous deficient state was obtained through cytological examination of normal sibs, which had only homozygous normal and heterozygous deficient chromosomes; through crosses of these latter plants to plants heterozygous for longer deficiencies, where the mutant types appeared only from unions of the two respective deficient chromosomes; through close if not complete linkage with the mutant *yg* located near the end of the short arm of chromosome 9; and through chromosomal examination within white sectors of sectorial plants.

Intercrosses among all 7 cases have shown that the 5 pale-yellow mutants are allelic and that the 2 white-seedling mutants are allelic to pale-yellow, with pale-yellow dominant to white. The 5 deficiencies giving rise to pale-yellow do not include the *yg* locus, whereas the 2 deficiencies giving rise to white seedlings may include this locus. The deficiencies giving rise to white seedlings are longer than those giving rise to pale-yellow seedlings.

although they have a deficient segment in common. This accounts for the allelic nature of the two mutants and the dominance of pale-yellow over white. The pale-yellow and white mutants represent typical Mendelizing mutants, which are associated with a state of homozygous deficiency. Dominance in these cases is an expression of the extent of the deficiency: no deficiency produces green seedlings, a short terminal deficiency produces pale-yellow seedlings, and a longer terminal deficiency produces white seedlings, with dominance expressed in this order.

The phenotypic effects of relatively long terminal homozygous deficiencies. Terminal deficiencies that include more than one chromomere do not give rise to functional pollen. Thus, the phenotypic effects of these deficiencies could not be studied by the direct method of selfing heterozygous plants. Instead, the variegation method, which produces sectors of tissue that are homozygous deficient, was introduced in these cases. This method utilizes the aberrant mitotic behavior of recently broken chromosomes, which, in the endosperm, continuously deletes segments from the arm of the chromosome which has the broken end. If the female gametophyte contributed 2 deficient chromosomes, and the male gametophyte contributed a chromosome 9 whose short arm terminated in a recently broken end, the developing endosperm could be sectorial for homozygous deficient tissues. The endosperm mutants *C* (aleurone color), *I* (inhibitor of aleurone color, allelic and dominant to *C*), *Sh* (*sh*, shrunken endosperm), and *Wx* (*wx*, waxy starch) were used to mark the chromosomes contributed by the two parents. The preliminary investigations on the effects of homozygous deficiencies on endosperm development may be summarized as follows: Endosperm development may be completely normal when homozygous deficiencies up to and including two terminal chromomeres are present. Beyond this region, only patches of such homozygous deficient tissue, surrounded by normal tissues, will develop. As the homozygous deficiency becomes progressively longer, the rate of development within the sector is reduced. Although the *C* locus may still be present, aleurone-color development progressively diminishes until only the rim of cells bordering normal cells shows color. Apparently, some substance or substances diffuse from the normal cells into these homozygous deficient cells, allowing them to develop normal aleurone color. This material, however, either does not diffuse beyond a layer several cells deep or is used up before deeper penetration occurs. Starch development occurs in all the patches of homozygous deficient cells except when the deficiency approaches the distal third of the short arm and includes the locus of *C*. In the latter case, relatively extensive growth of the homozygous deficient cells occurs; but, owing to lack of starch formation in these cells, a shrinkage leading to scar formation occurs after drying of the kernels.

To study the effects of various homozygous deficiencies on sporophytic tissues, the method of covering a deficiency with a ring-shaped chromosome may be utilized. Frequent losses of the ring-shaped chromosome during mitoses should produce cells that are homozygous deficient. Cells arising from these cells should produce sectors capable of expressing changes that could be related to the homozygous deficient state. Likewise, changes in constitution of ring chromosomes, which may delete segments from the ring, could produce sectors that are homozygous deficient for various segments within the limits of the full deficiency. Only two such plants

have been produced. Both plants were characterized by numerous sectors of white, pale-yellow, and yellow-green tissues. Although these sectors probably represent the expression of homozygous deficiencies, no conclusions will be drawn until this method receives more detailed and controlled analysis.

A deficiency of one-third of the short arm of chromosome 9 is relatively long, but none of these deficiencies have been cell lethal in any of the tissues studied. It is altogether possible that the observed effects of the homozygous deficiencies in the various tissues may be related to a few specific loci within the limits of the distal third of the short arm, rather than to the accumulative effect of a large number of such loci. This would be understandable if maize were a derived polyploid.